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TRPV1 and neuropeptide receptor immunoreactivity and expression in the rat lung and brainstem after lung ischemia-reperfusion injury

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ABSTRACT

Background: Activation of capsaicin-sensitive sensory nerves and TRPV1 present on them can ameliorate the ischemia-reperfusion injury in vital organs by evoking the release of neuropeptides including calcitonin gene-related peptide (CGRP) and substance P. However, the underlying changes in TRPV1 and neuropeptide receptor expressions, including calcitonin receptor-like receptor (CRLR) and neurokinin 1 receptor (NK1R), after lung ischemia-reperfusion injury (LIRI) remain uncharacterized.

Methods: Thirty-two male Sprague–Dawley rats were randomly and equally divided into sham (sham thoracotomy) and ischemia-reperfusion (occlusion of the left pulmonary hilus for 1 h followed by reperfusion for 4 h) groups. Blood gas levels were measured and histopathologic examination was performed. Left lung lobes and brainstem tissue samples were harvested for use in quantitative real-time PCR, Western blot, and immunohistochemistry to measure TRPV1, CRLR, and NK1R transcript and protein levels. Additionally, CGRP and substance P protein levels were quantified in the lungs using enzyme-linked immunosorbent assay.

Results: LIRI exacerbated blood gas exchange and increased the pulmonary tissue injury score. Furthermore, LIRI increased CGRP levels in the lung, TRPV1–immunoreactivity (ir) in the bronchiolar epithelium and smooth muscle of the pulmonary artery, and the intensity of neuronal CRLR-ir and NK1R-ir in the commissural nucleus of the solitary tract. Similarly, LIRI significantly elevated both transcription and translation of TRPV1 in the lungs and CRLR and NK1R in the brainstem.

Conclusions: Both transcription and translation of TRPV1 in the lungs and CRLR and NK1R in the brainstem of rats can be upregulated by LIRI in a rapid manner (within 5 h).

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Introduction

Lung ischemia-reperfusion injury (LIRI) is a common and severe postoperative complication that often occurs after lung transplantation, cardiopulmonary bypass, cardiopulmonary resuscitation, pulmonary embolism, and sepsis.[1,2] Recently, developments in surgical technology and their subsequent wide application have resulted in an inevitable surge in LIRI. The mechanisms underlying LIRI have been investigated, and the pathogenesis has been found to primarily be due to oxidative stress, inflammation, cellular apoptosis, intracellular calcium overload, and neurogenic inflammatory pathways.[3,4] Unfortunately, these underlying mechanisms are often systemic and complex and have not yet been completely elucidated.

Capsaicin-sensitive sensory nerves (CPSNs) are major pulmonary sensory nerves (i.e., nociceptive sensors) that play a vital role in regulating respiration under both physiological and pathophysiological conditions.[5] Transient receptor potential vanilloid subtype 1 (TRPV1) is a nonselective cation channel that is widely expressed on the central and peripheral terminals of CPSNs in the respiratory system. Activation of TRPV1 on CPSNs, such as by heat, protons, capsaicin, or other endogenous lipids known as endovanilloids, triggers the release of neuropeptides, including calcitonin gene-related peptide (CGRP) and substance P (SP), from both central and peripheral neuron terminals.[6] Importantly, activation of TRPV1 transmits nociceptive information to the brain and induces multiple autonomic reflexes.[6,7] CGRP binds to calcitonin receptor-like receptor (CRLR), whereas SP binds to neurokinin 1 receptor (NK1R). It has been shown that CGRP induces powerful vasodilation, particularly in the pulmonary artery.[8] Meanwhile, SP release mediates contraction of airway smooth muscles,[9] microvascular plasma leakage,[10] and mucus secretion.[11]

Recently, a number of studies in rats and rabbits have shown activation of TRPV1 may protect against myocardial,[12] pulmonary,[13] hepatic,[14] renal,[15] and cerebral[16] ischemia-reperfusion injury (IRI) by releasing neurotransmitters, in particular CGRP and SP. Nevertheless, these studies have focused on the changes in CGRP and SP levels or used agonist/antagonist or TRPV1 gene knockout animal models to characterize the effects of IRI on organ injury. The dynamic fluctuation of receptors of interest in peripheral and central sites subjected to LIRI remains uncharacterized. Therefore, the purpose of the present study was to investigate TRPV1, NK1R, and CRLR immunoreactivity and expression in rat lungs and brainstems after LIRI.

Materials and methods

Animals and grouping

Animal experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University. Thirty-two healthy male adult Sprague–Dawley rats (Animal Experimental Center of Clinical Medical College of Sichuan University) weighing 250-320 g were housed in an air-conditioned facility maintained at 21 ± 1°C with a 12-h light/dark cycle. Commercial food and water were consumed *ad libitum* and replenished daily. For the experiments, the 32 rats were randomly and evenly divided into sham and ischemia-reperfusion (IR) groups. After IRI induction or sham treatment, half of each cohort was killed, and the brainstem and left lung lobes were harvested for quantitative real-time PCR (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA). The brainstem and left lung lobes of the remaining 8 rats were collected and analyzed by immunohistochemistry (IHC) and Western blot (WB).

LIRI animal model

The LIRI rat model was performed based on previously published studies.[17] Briefly, the rats were anesthetized using an intraperitoneal injection of 3.0% pentobarbital sodium (60 mg/kg). Venous and arterial lines used for the administration of fluids and drugs and monitoring dynamic blood pressure were prepared and inserted into the rat. Anesthesia was maintained by continuous infusion of pentobarbital (0.05 mg/kg·min), vecuronium bromide (0.1 mg/kg·h), and saline (1 mL/h). After endotracheal tube insertion, each rat was ventilated with positive pressure using an HARVARDA-Inspira ASVP rodent ventilator (Harvard Apparatus, Boston, MA) with an inspiratory oxygen fraction (FiO_2) of 40% at a rate of 50-60 breaths/min and a tidal volume of 6-8 mL/kg. Systemic anticoagulation was confirmed by intravenous injection of heparin (250 U/kg). A left anterolateral thoracotomy was performed in the fifth intercostal space, and the pleura was exposed to allow the left hilus of the lung to be isolated. After stabilizing for 15 min, the tidal volume was decreased to 5-7 mL/kg, and the rate was increased to 70-75 breaths/min. Ischemia was induced by clamping the left pulmonary hilum, including the left main bronchus, artery, and vein, for 1 h, followed by reperfusion induced by removing the clamp and ventilating and reperfusing the left lung for 4 h. The sham group underwent the left thoracotomy with hilus dissection and was ventilated for 5 h. The thoracic incision was protected from evaporative losses using a covering of wet absorbent gauze. The body heat of the rats was maintained using a heating lamp at a rectal temperature within the normal range (36-37°C). At the end of the experiment, the rats were euthanized with a large dose of pentobarbital sodium (60 mg/kg, i.p.), and the left lung lobes and brainstem were harvested.

Monitoring of vital signs

The mean arterial pressure (MAP), heart rate (HR), and temperature of each rat were monitored continuously throughout the study, and values were recorded before ischemia, 1h after ischemia, and 1, 2, 3, and 4 h after reperfusion for each group.

Blood gas analysis

Arterial blood (0.3 mL) was collected from each rat before induction of ischemia as well as 0.5 h and 4 h after reperfusion, and then blood gas analyses were performed (ABL 800; Radiometer, Copenhagen, Denmark). Arterial partial pressure of oxygen (PaO_2) and arterial partial pressure of carbon dioxide ($PaCO_2$) were also recorded. The arterial-alveolar oxygen